

Prepared for the National Institutes of Health
National Institute of Neurological Disorders and Stroke
Division of Fundamental Neurosciences
Neural Prosthesis Program
Bethesda, MD 20892

Microstimulation of the Lumbosacral Spinal Cord: Mapping

NIH-NINDS-N01-NS-5-2331

Quarterly Progress Report #3

Period Covered: 1 April, 1996- 30 June, 1996

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ABC reagent from Vectastain kit purchased from Vector Laboratories (Burlingame, CA, USA). The ABC complex was visualized by the diaminobenzidine reaction, intensified by NiCl_2 . The sections were mounted on glass slides, washed, counterstained, coverslipped and viewed with brightfield optics.

No satisfactory labelling was obtained using 0.1% concentration of CT-b as a retrograde transganglionic marker. This result was unexpected because the same volume and dilution of CT-b injected into trachea or low esophageal sphincter resulted in intense and specific labeling of motor and sensory vagal systems innervating these end organs. Taken together, these results suggest that successful detection of specific labeling is strongly dependent on the site of injection, the volume of injection, and the concentration of the tracer. We will repeat these experiments using varying volumes of 1% CT-b.

B. Psuedorabies Virus Experiments in Male Rats

As described in QPR Nos. 1 and 2, psuedorabies virus (PRV) is a trans-synaptic retrograde neuronal tracer. We continued our tracing studies in male rats to fill out the range of post-injection survival times (3-8 days). This will allow us to speculate on the putative order of labeled neurons as higher order neurons will only be labeled in animals with longer survival times.

C. Tracing Experiments in Cats using Multiple Retrograde Tracers

We conducted double tracer studies of the innervation of the pre-prostatic urethra in adult male cats. The objective of these experiments is to determine the location and extent of the neurons in the spinal cord which innervate the proximal urethra.

Cats were anesthetized with xylazine (20mg/kg, SQ) and Ketamine (15 mg/kg, IM), intubated and maintained on gaseous Halothane (1-2%) in O_2 . A catheter was inserted in the cephalic vein body temperature saline (10 cc/kg/hr) was administered throughout the procedure. Body temperature was maintained with a heating pad, and heart and respiratory rate were continuously monitored. The lower abdomen was shaved, washed with alcohol, and scrubbed with povidone iodine. The area was surrounded with sterile drapes and covered with a sterile incise drape. The pre-prostatic urethra was exposed through a midline incision just rostral to the pubic symphysis using aseptic technique. The retrograde tracer Fluorogold (100 μl of 4% solution) was injected into the urethral wall 1-2 cm proximal to the prostate and the retrograde tracer cholera toxin β -subunit (150 μl of 1% solution) was injected into the urethral wall 3-4 cm proximal to the prostate. Based on the results obtained in our rat studies we increased the CT-b concentration by ten times, from 0.1% to 1.0%. Tracer solution was absorbed from around the puncture site during injection with sterile surgical spears, and after injection the area was swabbed and repeatedly rinsed with sterile saline. The muscle and fat layers were closed with 4-0 chromic gut sutures and the skin closed with 4-0 Prolene. Oxacillin sodium, a broad spectrum antibiotic, was administered post-operatively (100 mg/kg), and the animals were monitored closely until fully recovered from anesthesia.

These animals are presently in their post-injection survival period. After 7-10 days they will be re-anesthetized, perfused via the aorta with Paraformaldehyde, and the urethra and spinal cord will be removed for processing.

II. Functional Anatomy of the Urethra

We consider a thorough understanding of the urethral pressure profile (UPP) a necessary precursor to assessing systematically the effects of spinal cord microstimulation on urethral pressure. Towards this end, we conducted experiments to determine the anatomical structures that give rise to specific pressures along the length of the urethra.

Methods

Seven experiments were conducted on adult male cats. In two experiments urethral pressure profiles (UPPs) were measured using a saline filled tube connected to a solid state pressure transducer. The poor fidelity and offset inaccuracies associated with this technique prompted us to purchase a 2 channel solid state micromanometer mounted on a solid 5F catheter

in this region which is expected to increase pressure on the catheter. There is also an increase in the circumurethral muscle mass (urethralis, ischiourethralis, bulboglandularis, and ischiocavernosus) at this level, and somatic spiking could also be observed here. Distal to the base of the penis a second peak was observed which appears to correspond to the level of the bulbospongiosus muscles. The pressures are generally lower in the penile urethra, although there was often an increase towards the tip of the penis as the circumference of the urethra diminished [Agnew et al., 1995].

Electrical activation of the pudendal nerve generated large increases in urethral pressure. In this example (fig. 2) pressure increases due to activation of somatic muscle were observed from the level of the prostate to the level of the penile urethra. This corresponds well to the regions found in histological sections to contain striated skeletal muscle. In some stimulation trials the apparent functional length of the urethra is shorter than in the passive trials (figs. 2A and C). In these trials the activation of the pelvic floor musculature via the pudendal nerve generate a "pumping" motion that tended to push the catheter out of the urethra faster than the pull rate over the last several cm of the urethra.

We found that the profile of the stimulus evoked pressures was strongly dependent on the stimulus frequency. In this example stimulation with a continuous 2 Hz train generated pressures from the level of the prostate to the level of the penile urethra. However, stimulation with a 20 Hz burst stimulus (ON for 1 s/OFF for 1 s) only generated pressure increases from the caudal prostate to the base of the penis. One factor that may contribute to this difference is pressures transmitted from activation of other non-urethral pelvic musculature.

Another potential explanation for the frequency dependence of pressure profiles may lie in the differences observed during afferent and efferent stimulation. Urethral pressure profiles were generated by stimulation of either the proximal or distal stumps of transected pudendal nerve branches. An example in which the posterior branch (innervating external anal sphincter) of the right pudendal nerve was transected is shown in fig. 3. Activation of the distal (efferent) end of the branch generated little change in the profile of pressures along the urethra (figs. 3A,B). However, activating the central (afferent) end of the branch generated pressure profiles akin to those observed during whole nerve stimulation (fig. 2). The pressure profiles generated by central stimulation were strongly dependent on the stimulus frequency, in both their rostrocaudal extent and their magnitudes (figs. 3C-J). Lower stimulus frequencies generated larger pressures over a greater length of the urethra. These results suggest that at least some of the stimulus evoked pressures presented in fig. 2 were due to activation of pudendal afferent nerve fibers which in turn activated pudendal efferents.

In addition spatial variations, the pressure responses also differed in their temporal patterns at different stimulus frequencies. Figure 3A shows the pressures generated in the urethra ~2 cm caudal to the prostate at different stimulus frequencies. Because the catheter was withdrawn slowly (1mm/sec) this is essentially a quasistatic measurement with respect to position. At the lowest stimulus frequency (2 Hz) the response follows one-to-one with the stimulus and maintains a constant amplitude. At higher frequencies (5 Hz, 10 Hz), the response still follows one-to-one, but the magnitude falls dramatically between the first and second stimulus, and continues to fall in response to the 10 Hz stimulus. With the highest stimulus frequency (20 Hz) only a single response is observed and the pressure returns to baseline.

A similar dependence of response properties on stimulus frequency has also been observed during microstimulation of the sacral spinal cord. Figure 3B shows the urethral pressures evoked by microstimulation with either a 2 Hz or a 20 Hz stimulus train. The electrode that generated these pressures was positioned in the dorsal horn of S2, approximately 400 μ m below the cord surface. The pressure responses followed one-to-one with the 2 Hz stimulus and had a relatively invariant amplitude. However, the response to the 20 Hz stimulus fell off rapidly after the first 3 pulses of the train. In light of the above results with stimulation of pudendal afferents, these data support the previous assertion that electrodes in the dorsal aspect of the spinal cord evoke genitourinary responses (both bladder and urethra) via indirect means. That is, they activate electrically either afferent axons or propriospinal neurons interposed between afferent fibers and efferent neurons.

traces shown in fig. 5C which illustrate the transmitted pressure wave beginning in the bladder and passing down the urethra, first past the proximal sensor, and then past the distal sensor.

This post-stimulus flow is by no means a novel observation, and is the basis for a commercially deployed genitourinary neural prosthesis that relies on the temporal response differences between smooth and striated muscle to achieve post-stimulus voiding [Brindley et al., 1982]. However, this finding is important in that it demonstrates that in the absence of stimulus evoked activation of the urethra, flow can be achieved to at least the level of the penile urethra (the distal catheter was positioned ~3 cm from the external meatus). In other words, in this experiment the passive resistance to flow provided by the basal tone of the urethra would not obstruct flow resulting from the bladder pressures we were able to generate.

IV. Measurement of Knee Torques Generated by Microstimulation of the Lumbar Spinal Cord

The objective of these experiments is determine the range of torques that can be generated at the knee joint by microstimulation of the lumbar spinal cord. A secondary objective in these experiments is to gain experience using silicon multi-site microprobe electrodes (provided by the University of Michigan) for microstimulation of the spinal cord.

Methods

The animal preparation was as described above in III. The knee was instrumented with a strain gage beam to measure the isometric torque generated by stimulation (see QPR No. 2). The lumbar segments were identified anatomically and functionally by recording the responses to stimulation of the spinal roots with a hook electrode. Vertical penetrations were made in the L5 and L6 segments at different mediolateral locations. All stimuli were charge balanced biphasic regulated current 100 μ s pulses. In this experiment a 5-site silicon microprobe electrode was used, with each site acting as a separate monopolar point source and a subcutaneous needle serving as the return electrode. The sites were spaced 200 μ m apart along the electrode shank, and had impedances of ~75 k Ω . Responses to 1-5s trains of various amplitude current pulses at 2-20Hz were recorded at different depths along each penetration.

Results

We encountered difficulty in penetrating the surface of the spinal cord with the silicon microprobe electrodes. Rather than penetrating the spinal cord surface, the probes would bend when advanced by a manual micromanipulator. It may be possible to improve penetration by using a rapid step change in position as provided by a motor driven manipulator. We found that we could grasp the probe with a small pair of forceps (tips covered with small caliber silicone rubber tubing) and start the penetration manually. After the initial penetration, we were able to advance the probe with the manipulator.

Figure 6 shows the torque responses generated along a vertical dorsal-to-ventral penetration in rostral L6, ~500 μ m medial to the dorsal root entry zone. Note that the torque responses are not fused in response to a 20 Hz stimulus train. As reported in QPR No. 2, electrode locations deep in the ventral aspect of the cord generated motor responses at the knee. Along this penetration all torques were in the extension direction, although without recordings of electromyograms, co-contraction cannot be excluded. It is clear, particularly at location 4, that the response magnitude was graded both by the stimulus amplitude and the site that was used. For example, 200 μ A applied at site 5 generated approximately the same response as 100 μ A at site 4 and 50 μ A at site 1.

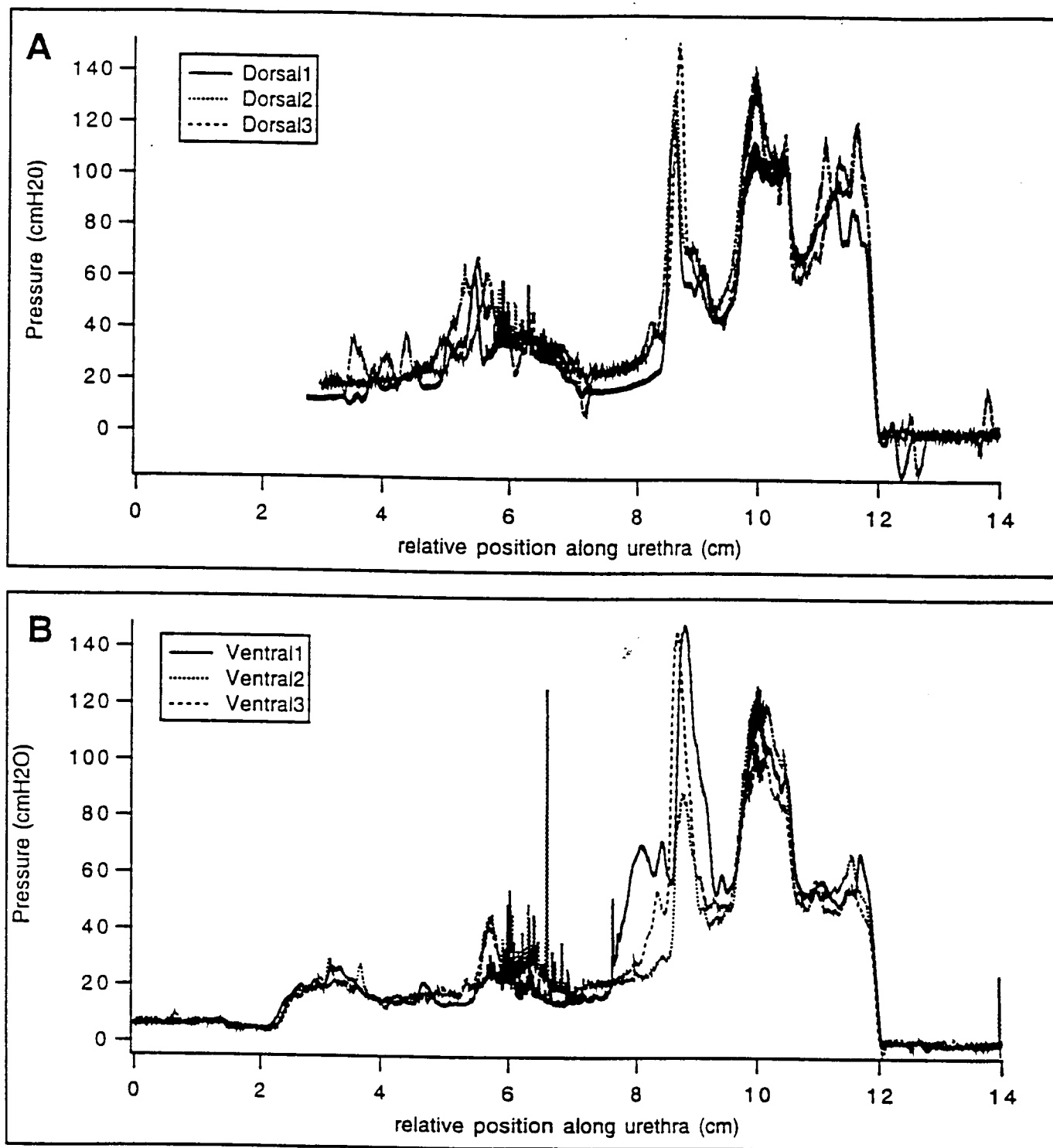


Figure 1: Passive urethral pressure profiles (3 trials) measured in a male cat anesthetized with α -chloralose. A 2-channel solid state pressure transducer was withdrawn along the urethra (1mm/sec) and the pressure was recorded by a dorsally (A) and ventrally (B) oriented sensor. The vesico-urethral junction was located at ~2 cm on the relative scale, and thus in these trials the dorsal sensor had an initial position in the pre-prostatic urethra, rather than in the bladder. The exit of the sensors from the tip of the penis to atmosphere was located at ~12 cm on the relative scale. Spiking of somatic musculature was seen caudal to the prostate (6-7 cm) as well as in the region of the bulbospongiosus (10-11 cm).

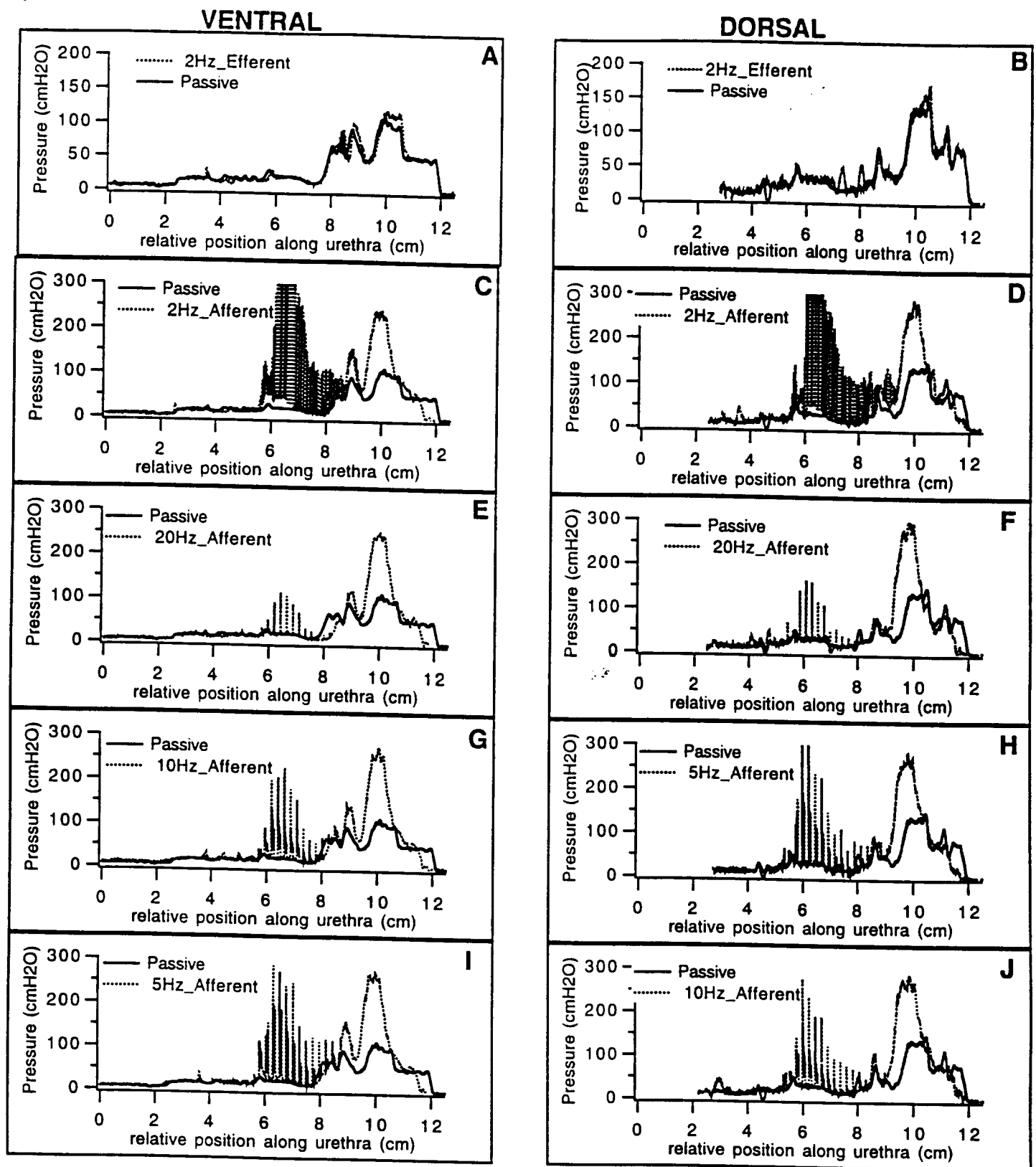


Figure 3: Passive (*solid lines*) and active (*dotted lines*) urethral pressure profiles measured as in fig. 1 in the ventral (left) and dorsal (right) directions. The active profiles were generated by electrically stimulating either the distal (A, B "efferent") or proximal (C-J "afferent") stump of the transected posterior branch of one pudendal nerve with a hook electrode while the catheter was withdrawn. Again note that the apparent functional length of the urethra is shorter in the active trials than in the passive trials as a result of the "pumping" generated by the activity in the pelvic floor muscles.

Figure 5: Physiological responses in the genitourinary system generated by microstimulation of the sacral spinal cord. These responses were evoked along a vertical, dorsal-to-ventral penetration in mid-S2, ~250 μ m lateral to the dorsal root entry zone. All stimuli were charge balanced biphasic regulated current 100 μ s pulses applied at 20 Hz for 1 s. As in previous experiments, large evoked bladder pressures (baseline pressure ~ 15 cmH₂O) were generated over a widespread region of the cord (A). Large transient pressures were also evoked in the proximal and distal urethra (baseline pressure ~27 cmH₂O) (B, D). These responses appeared to be mediated by somatic skeletal muscle as they had a rapid onset and offset (C) and the pressure fell from a peak at stimulus onset to a lower average value during the 1s stimulus train (B, D). The pressure waves (C) recorded in the bladder, proximal urethra, and distal urethra indicate that fluid flowed from the bladder to at least the level of the distal pressure sensor.

Figure 6. Isometric extension torques generated by microstimulation of the lumbar spinal cord. These responses were evoked along a vertical, dorsal to ventral penetration in rostral L6, ~500 μ m medial to the dorsal root entry zone. All stimuli were charge balanced biphasic regulated current 100 μ s pulses applied at 20 Hz for 1 s. In this experiment a 5-site silicon microprobe electrode was used, with each site acting as a separate monopolar point source and a subcutaneous needle serving as the return electrode. Note that the torque responses are not fused in response to a 20 Hz stimulus train.